

## APPENDIX I:

CLAIM AMENDMENTS:

Kindly amend Claims 27 and 33 as indicated in the following listing of the claims:

1. - 26. (*canceled*)

27. (*currently amended*) A process for the microbiological oxidation of a substrate compound having an N-, O- or S-heterocyclic mono- or polynuclear aromatic moiety, which process comprises oxidizing at least one aromatic C-H group of the heterocyclic aromatic moiety by

a1) culturing a recombinant microorganism which expresses a cytochrome P450 monooxygenase of bacterial origin in a culture medium, in the presence of an exogenous or intermediately formed substrate; or

a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase of bacterial origin; and

b) isolating the oxidation product formed or a secondary product thereof from the medium, and

wherein the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having the amino acid sequence according to SEQ ID NO:2 by mutation,

and the monooxygenase has a ~~functional~~ mutation which consists of a functional mutation in ~~at least one, two or all~~ of sequence positions 74, 87 and 188, whereby

Phe87 is replaced by Ala, Val or Leu,

Leu188 is replaced by Asn or Gln, and/or

Ala74 is replaced by Val or Gly.

28. (*previously presented*) The process as claimed in claim 27, wherein the exogenous or intermediately formed substrate of claim 27, alternative a1), or the substrate contained in the reaction medium of claim 27, alternative a2) is selected from optionally substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds.

29. (*canceled*)

30. (*previously presented*) The process as claimed in claim 27, where the mutant has one of the following mono- or polyamino acid substitutions:

- a) Phe87Val;
- b) Phe87Val and Leu188Gln;
- c) Phe87Val, and Leu188Gln, and Ala74Gly.

31. (*previously presented*) The process as claimed in claim 27, wherein the exogenous substrate is at least one compound selected from unsubstituted or substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds, the exogenous substrate is added to a medium and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of approximately 20 to 40°C and a pH of approximately 6 to 9, where the substrate-containing medium additionally contains an approximately 10- to 100-fold molar excess of reduction equivalents based on the substrate.
32. (*previously presented*) The process as claimed in claim 31, wherein the exogenous substrate is a compound selected from indole, 1-methylindole, acridine, 6-methyl- or 8-methylquinoline, quinoline and quinaldine.
33. (*currently amended*) The process for the microbiological production of indigo and/or indirubin, which comprises
- a1) culturing a recombinant microorganism which produces an indole-oxidizing cytochrome P450 monooxygenase in a culture medium, in the presence of exogenous or intermediately formed indole; or
  - a2) incubating an indole-containing reaction medium with an indole-oxidizing cytochrome P450 monooxygenase; and
  - b) isolating the oxidation product formed or a secondary product thereof from the medium,
- wherein the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having the amino acid sequence according to SEQ ID NO: 2 by mutation,
- and the monooxygenase has a ~~functional~~ mutation which consists of a functional mutation in ~~at least one~~, two or all of sequence positions 74, 87 and 188, whereby
- Phe87 is replaced by Ala, Val or Leu,
  - Leu188 is replaced by Asn or Gln, and/or
  - Ala74 is replaced by Val or Gly.
34. (*previously presented*) The process as claimed in claim 33, wherein the indigo and/or indirubin obtained, which was produced by ox-

idation of intermediately formed indole, is isolated from the medium.

35. (*previously presented*) The process as claimed in claim 34, wherein the indole oxidation is carried out by culturing the microorganisms in the presence of oxygen at a culturing temperature of approximately 20 to 40°C and a pH of approximately 6 to 9.
36. (*canceled*)
37. (*previously presented*) The process as claimed in claim 35, where the monooxygenase has at least one of the following mono- or poly-amino acid substitutions:
- a) Phe87Val;
  - b) Phe87Val, Leu188Gln; or
  - c) Phe87Val, Leu188Gln, Ala74Gly.
38. - 49. (*canceled*)
50. (*previously presented*) The process as claimed in claim 27, wherein the functional mutation occurs in the amino acid sequence position 87, or in the positions 87 and 188, or in the positions 87, 188 and 74, whereby
- Phe87    is replaced by Ala, Val or Leu,  
Leu188   is replaced by Asn or Gln, and  
Ala74    is replaced by Val or Gly.
51. (*previously presented*) The process as claimed in claim 33, wherein the functional mutation occurs in the amino acid sequence position 87, or in the positions 87 and 188, or in the positions 87, 188 and 74, whereby
- Phe87    is replaced by Ala, Val or Leu,  
Leu188   is replaced by Asn or Gln, and  
Ala74    is replaced by Val or Gly.